ROTHAMSTED RESEARCH

Report for 1982 - Part 1

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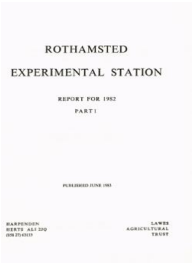

Soil Microbiology Department

J. E. Beringer

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SOIL MICROBIOLOGY DEPARTMENT

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Introduction

The Department is concerned with the study of soil microorganisms that have beneficial effects on plant growth. Work on biological nitrogen fixation and vesicular-arbuscular mycorrhizal fungi is proceeding satisfactorily and will continue. More emphasis is being put into developing new and improved methods for handling and identifying soil micro-organisms, techniques that are essential for future departmental research.

Refurbishing of the Department started in the winter of 1981–82 and was completed by October, 1982. The refurbishing has involved the complete redesign of the space available to the Department, and the rewiring, flooring, plumbing and refitting of most laboratories. This has provided more space and a cleaner and safer environment in which to work. We are grateful to the E & MS Department and Mr R. Taberer in particular for the efficient way in which the work was organized.

As in previous years, we are indebted to the ODA and NRDC for providing grants that have enabled us to increase the range of problems studied within the Department. During 1982 we were in receipt of two grants from each agency supporting a total of four HSOs and four ASOs. We are particularly grateful to the ODA for providing a Micromass mass spectrometer to enable us to develop methods for measuring biological nitrogen fixation using ^{15}N compounds.

We are pleased to report that the production of *Rhizobium* inoculants in the UK will be resumed in 1983, after a break of many years. The inoculants will be produced by a

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company (New Plant Products Limited, NPPL) which has been set up by the British Technology Group. Members of the Department have been involved in setting up the company and will have a continuing role in offering advice in the future. We hope that in future NPPL will provide a facility for the production of inoculants containing other groups of microorganisms. Our association with this company should facilitate the commercial exploitation of research in the Department and will provide a firm basis for the development of new strains of microorganisms for inoculation purposes.

Physical studies on soil microorganisms

Sampling the soil microflora. Physical and chemical methods have been developed to obtain samples of microorganisms from soil. These techniques produce a suspension of living cells which is substantially free from contamination by soil debris. Density gradient centrifugation has allowed limited fractionation of such suspensions; the concentration of various fractions has been measured by flow cytometry using fluorochromes specific for DNA or protein. (Macdonald and Sen)

Differentiation of microorganisms by pyrolysis techniques. Anoxic thermal degradation of intact microbial cells produces a mixture of degradation products (the pyrolysate). The relative proportions of definable (but not necessarily identifiable) pyrolysis products are characteristic of the microorganism pyrolysed. This approach may be useful in differentiating physico-chemically defined populations of microorganisms prepared by the techniques outlined above, and is being applied to the differentiation of *Rhizobium* isolates. The standard microbial identification tests used for this purpose are time-consuming and often given an incomplete answer because isolates are often phenotypically very similar. Differentiation of certain species and strains has been achieved by direct probe mass spectrometry (DPMS; Macdonald and Dye with Mudd, Insecticides Department) and isothermal gas-solid chromatography (IGSC; Dye, Macdonald and Gostick). Certain isolates may be differentiated readily by applying canonical variates analysis to the 'fingerprints' derived from analyses of pyrolysates. Others are more difficult to distinguish and work is being done (1) to define the limits of usefulness of DPMS and IGSC and (2) to find out what modifications of techniques are necessary to increase resolution. (Macdonald and Dye)

Rhizosphere microbiology

Microbiology of wheat roots. Bacteria were isolated from roots of wheat grown consecutively for 3 or 6 years. Isolates were tested for their effects on germination, growth and dry weight of wheat seedlings grown in partially sterilized Kettering loam. Of cultures from the third crop, 25% had some beneficial and 14% some deleterious effects, compared with 50 and 2% respectively, from the sixth crop. These effects were not observed on seedlings grown in unsterile Broadbalk field soil.

Healthy segments of wheat seedling roots were incubated for 3 days in suspensions of mixtures of bacteria isolated from the rhizosphere. All root segments incubated with bacteria from the third crop became discoloured compared with only 14% incubated in suspensions from the sixth crop. When individual isolates were tested, more from the third crop (58%) than from the sixth (48%) caused discoloration. Seedlings were germinated and grown for 7 days in Petri dishes containing either the mixtures of bacteria or water. The seedlings grew less well in suspensions of isolates from the third crop than in the sixth or water.

Isolates were categorized by their nutritional requirements: simple if they grew in a mineral medium, complex if they required an addition of casein hydrolysate to the

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mineral medium. More bacteria from the third than the sixth crop were 'simple' and grew more rapidly in the mineral medium. Hildebrand and West (1941) regarded bacteria with complex requirements as those which occurred in a normal healthy rhizosphere and the 'simple' bacteria as abnormal and associated with roots where disease develops. The abnormal types could be the cause of much discoloration on roots and contribute to the general susceptibility of the plant to fungus attack. In the present experiments bacteria causing discoloration of root segments belonged to both nutritional groups (Hildebrand and West, *Canadian Journal of Research C* (1941), **19,** 183–198). (Brown and Gibson)

Comparative anatomical studies of wheat roots invaded by *Gaeumannomyces graminis* var. *tritici* (Ggt), *Phialophora radicicola* var. *graminicola* (Prg) and vesicular-arbuscular endophytes (VAM). These studies have continued on whole segments of roots or transverse and longitudinal sections taken from infected areas. Inocula of Ggt and Prg were placed near root tips at 3 or 8 cm from the seed. The speed of invasion by both fungi was similar once penetration had occurred. Sections of roots were cut over a 3 week period, and these showed that some cells of all layers of the cortex of control uninfected roots retained nuclei for this time, but loss started in the outer three layers after 10 days and progressed at an increasing rate as roots aged. Most nuclei were in cells of the two inner layers. In infected roots nuclei were associated with hyphae in some cells of the outer cortex of young roots, but were not observed after 10 days. Nuclei were always present in infected cells of the two inner layers but at a lower frequency than in control roots.

Associations between nuclei and mycorrhizal hyphae were studied on 28 day-old roots. Infected cells were concentrated in the inner cortex. In approximately 40% of the transverse sections examined the same proportion of nucleate cells were noted in the epidermis and each layer of the cortex.

Observations of healthy nuclei suggest that cells of the epidermis and outer cortex remain alive for a short time after invasion of the root by Prg and Ggt and for a longer time after invasion by VAM. Nuclei are associated with all three fungi in the cortical layers next to the endodermis. Observations with material prepared for electron microscope studies should give more information on the longevity of cells invaded by the fungi. (Brown, Hepper, Chandler and Gibson with Hornby, Plant Pathology Department)

Vesicular-arbuscular mycorrhiza

White clover in Welsh hill grasslands. New experiments on the possibility of improving the establishment of clover introduced directly into the native turf after flail-mowing were initiated at Pwllpeiran EHF in cooperation with the Welsh Plant Breeding Station, Aberystwyth. Four treatments were compared: M (inoculated with a mixture of *Glomus asciculatum* 'E3', *Glomus mosseae* 'YV' and *Glomus caledonium* 'LAM'); P (superphosphate at 90 kg P ha^{-1}); M+P; and C (controls with no M or P). In mid-May, clover was introduced either as young transplants, or as seeds in a pelleted inoculum about 1 cm diameter.

Both grass and clover grew well. By mid-July there were strong visual differences between treatments, clover establishment being poor in C, excellent in M+P and intermediate in M and P plots. Results for transplants and pellets were similar. Native VA endophytes infected less than the introduced ones. Dry matter production of clover harvested in late September from the plots with pellets was 51, 50, 214 and 14 g per plot for treatments M, P, M+P and C, respectively. This optimum combination of mycorrhizal inoculation and superphosphate application agrees with earlier results from Pwllpeiran at sites where the indigenous mycorrhizal endophytes were less effective than

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the selected introduced ones and where the peaty soil did not become too wet. Clearly it is possible to improve greatly the establishment of white clover in competition with the native vegetation of upland grasslands under certain conditions, and we are now in the process of elucidating these conditions. (Hayman)

Maize at Grassland Research Institute. Previous observations suggested that an active indigenous population of mycorrhizal fungi helped to overcome P-deficiency in field-grown maize (see *Rothamsted Report for 1981*, Part 1, 210). Therefore, a general biocide (dazomet) was used to suppress this population so that the role of added mycorrhizal fungi could be tested. Four treatments were compared: M (with mycorrhiza, primarily *Glomus mosseae*); P (with superphosphate at 13 kg P ha^{-1}); M+P (with mycorrhiza and superphosphate); and C (controls with no M or P). Soil phosphate levels averaged 34 ppm bicarbonate ($NaHCO_3$) soluble P. By July there was considerable infection in the M and M+P treatments, but infection built up only very slowly in the uninoculated treatments. At harvest, however, there were no significant differences in dry matter production between all four treatments. It appears that although dazomet inhibited indigenous endophytes during the main period of plant growth, it also enhanced early crop growth, thereby masking the anticipated benefits from mycorrhizal inoculation. (Hayman, Grace, Spokes, O'Shea and Dr A. Schubert, University of Turin)

Saprophytic growth of VA mycorrhizal fungi. Warner and Mosse (*Transactions of the British Mycological Society* (1980), **74,** 407–410) found that VA mycorrhizal fungi have the ability to grow saprophytically in soil and establish a base from which they can infect a host root. We have found that no saprophytic growth and colonization are possible in a sand and grit mixture unless this medium has been supplemented by the addition of peat or the organic matter fraction isolated from soil. The infective propagules formed during saprophytic growth have not been identified but they are capable of survival in soil, in the absence of a plant, for up to 50 days. (Hepper and Warner)

VA mycorrhiza and fungal pathogens of plant roots. The effect of VA mycorrhiza on the severity of fungal diseases of plant roots was tested in several combinations. Pre-infection of seedlings with a mixture of *Glomus mosseae* and *G. caledonium* was found not to influence the development of *Verticillium* wilt (*V. albo-atrum*) of tomato and lucerne, *Fusarium* root rot (*F. oxysporum*) of lucerne, or red core disease (*Phytophthora fragariae*) of strawberry. Mycorrhizal infection in tomato roots decreased with increasing inoculum levels of *Verticillium*, viz. from 45% of the root length in healthy plants to 29% in plants inoculated with the highest level of the pathogen. (Dr E. Bååth, University of Lund, and Hayman)

Effect of host nutrition on VA mycorrhizal infection. Lettuce plants growing in a sand+grit mixture supplied with nutrient solution had less mycorrhizal infection (expressed as percentage of, or total, root length infected) at low levels of applied calcium. With *Glomus mosseae* infection levels were 7% at 1 mg Ca^{2+} l^{-1} compared with 44% at 40 mg l^{-1}. This difference was not attributable to any changes in the root tissue phosphorus levels. (Hepper and O'Shea)

Mycorrhizal infection in lettuce (expressed in terms of yield of glucosamine, percentage, or total, root length infected) increased as nitrate was supplied to the plants. The most important factor governing infection appeared to be the ratio of nitrogen to phosphorus in the plant nutrient solution—a high N:P ratio favouring infection. Plants which had a high root phosphorus content were immune to infection; those with normal phosphorus

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levels (*c.* 0·1%) only became well infected if their root nitrogen level was above 1·5%. (Hepper)

The effects of ammonium-N and nitrate-N on mycorrhizal infection (*Glomus caledonium*) of chives (*Allium schoenopraeum*) in a soil + sand mixture. With no added P (bicarbonate-soluble soil P 5·6 ppm), the percent root infection was not affected by increased N except for a decrease in infection at the supra-optimal N level. Levels of N used were 0, 50, 100 and 250 ppm for both NH_4^+ and NO_3^-.

With small additions of P (amending soil P to 16 or 26 ppm bicarbonate-soluble P) infection increased with increasing N until a supra-optimal level was reached, when infection decreased. A supra-optimal P levels (>48 ppm bicarbonate-soluble soil P), adding nitrogen decreased infection at all levels of N.

Growth responses to inoculation were recorded at 5·6 and 16 ppm bicarbonate-soluble P, at all levels of N. (Spokes and Dr E. Bååth, University of Lund)

Effects of nitrogen on mycorrhizal infection. Onion plants, mycorrhizal with *Glomus mosseae*, in a nitrogen-deficient soil of moderate phosphate fertility (35 ppm bicarbonate-soluble P) grew well when supplied with N as $Ca(NO_3)_2$, but not as NH_4NO_3. By contrast, plants given phosphate instead of mycorrhiza grew equally well with either form of N. Controls given neither phosphate nor mycorrhiza were P-deficient and showed no response to either form of N. With small additions of P the mycorrhizal plants grew slightly better with NH_4NO_3 than with $Ca(NO_3)_2$. It seems that the influence of P on the differential effects of the two forms of nitrogen on plant growth operated through the plant rather than through the fungus in the soil. (Wang and Hayman)

The effect of fungicide-coated seed on mycorrhizal establishment. This was studied in steam-sterilized low P soil (5·6 ppm bicarbonate-soluble P). Onions (*Allium cepa*) commercially coated with either 'Rovral' (iprodione), thiram or 'Benlate' (benomyl) were compared with non-treated seed for time of establishment and subsequent development of mycorrhizal infection. No differences were found. However, when the three fungicides were mixed into the soil at the commercially recommended rates, mycorrhizal infection was suppressed for the duration of the experiment. (Spokes, Dr D. Kandasamy, Tamil Nadu Agricultural University, and Hayman)

The effect of plant density and soil volume on VA mycorrhiza. Onion seedlings (1 to 12 per pot) were grown in 7·5, 10 and 12·5 cm diameter pots with a mixed mycorrhizal inoculum (*Glomus mosseae* and *G. caledonium*) or with no inoculum (controls) in a low phosphate (8 ppm bicarbonate-soluble P) arable soil. After 11 weeks mycorrhizal plants were all larger (on a dry weight basis) than the non-mycorrhizal controls, an effect which increased markedly with increasing pot size and decreasing plant density. The root/shoot ratio was always lower in the mycorrhizal plants; it decreased with increasing plant density irrespective of the mycorrhizal status, whereas no effect of pot size (total soil volume) was found. Mycorrhizal infection (percentage root length infected) decreased with increasing root density. (Dr E. Bååth, University of Lund, and Hayman)

Behaviour of VA mycorrhizal endophytes in soil amended with different amounts of phosphate. Seven endophytes were inoculated separately on to onion plants in P-deficient (8 ppm bicarbonate-soluble P) steam-sterilized soil amended with five rates of $Ca(H_2PO_4)_2.H_2O$. Mycorrhizal infection was usually decreased by increasing additions of P, but the rate of root colonization and the infection plateau varied with different endophytes. Plant growth responses to mycorrhiza were largest at low P with

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Glomus mosseae 'YV', at low-medium P with *Glomus fasciculatum* 'E3', at medium P with *Glomus macrocarpum*, *Glomus epigaeum* and *Gigaspora margarita*) and at most P levels with *Glomus caledonium*. The correlation between infection and growth response was higher for some endophytes than others. It was concluded that analyses of the VA symbiosis with a given endophyte cannot always be used to generalize about VA mycorrhizal fungi. (Dr A. Schubert, University of Turin, and Hayman)

Soil pH preference of different VA mycorrhizal endophytes. The infectivity and ability to improve growth of alpine strawberry seedlings in sterilized (γ-irradiated), low-phosphate soils (4 and 8 ppm bicarbonate-soluble P) limed to different pHs were assessed for nine endophytes. At pH 4 *Glomus clarum* greatly stimulated plant growth, but the other endophytes except *Glomus fasciculatum* 'E3' had no effect. The most efficient endophytes at pH 5 were 'E3', *Acaulospora laevis* and *G. clarum* (in that order). The largest plants were those growing at pH 7 and inoculated with *Glomus epigaeum*; *A. laevis* and *G. clarum* were ineffective at this pH. The best endophytes at pH 6 and pH 7 were *G. epigaeum*, *Glomus mosseae* 'YV', *Glomus caledonium* and *Gigaspora heterogama*. *Glomus macrocarpum* was generally ineffective. Most endophytes infected well at all pHs, even where they did not enhance plant growth. Thus, although in studies of this nature the ability of a plant to grow at a particular pH is important, these results show clearly that different endophytes vary enormously in their symbiotic effectiveness at different soil pHs. (Dr M. Tavares, Federal University of Minas Gerais, and Hayman)

Optimum VA endophyte—clover combinations at different levels of phosphate and light. The symbiotic performances of three endophytes (*Glomus fasciculatum* 'E3', *Glomus mosseae* 'YV' and *Glomus caledonium* 'LAM') on white clover were compared in a sterilized (γ-irradiated) low phosphate soil (8 ppm bicarbonate-soluble P). *Rhizobium trifolii* R221 was added to all plants. $Ca(H_2PO_4)_2.H_2O$ was applied at four rates and there were two light intensities, 16 700 and 7600 lux. 'E3' improved plant growth most, especially at the lower levels of P. 'LAM' gave no growth responses. Mycorrhizal infection was decreased by high P levels (percentage root length infected) and by low light intensity (fewer vesicles). The root nodules on 'E3'-inoculated plants were larger than those on uninoculated plants given much P, suggesting a direct stimulation of *Rhizobium* activity by the VA fungus. (Wang and Hayman)

Changes in endophyte efficiency in different soils. Seven endophytes were tested for their efficiency in stimulating growth of onions in three soils. *Glomus caledonium* 'LAM', *Glomus* spp. from Denmark (DEN), *Glomus clarum* from Germany (GOT) and *G. clarum* from Rothamsted (GCRM) were tested in soil S (Sawyers, 8 ppm bicarbonate-soluble P, 0·1% N). 'LAM,' *Glomus epigaeum*, a *Glomus* species from Spain (GSS) and DEN were tested in Soil A (Ashridge, 40 ppm bicarbonate-soluble P, 0·2% N). *G. epigaeum* GOT, GCRM and *Acaulospora laevis* were rested in soil W (Woburn 35 ppm bicarbonate-soluble P, 0·05% N). DEN infected fastest and produced a visible growth response after only 2 weeks in both soils A and S. 'LAM' was the next most efficient, then *G. epigaeum*. After 14 weeks all endophytes except GCRM had produced good infections and increased plant dry weight over the controls in soils A and S, DEN and 'LAM' isolates increasing dry weight several fold. In soil W the four endophytes tested did not improve plant growth although roots were well infected. (Grace)

The effect of heat on both soil- and peat-grown mycorrhizal inoculum. This was tested with the following endophytes: *Glomus mosseae*, *G. caledonium*, *G. fasciculatum* and *Acaulospora laevis*. Heating damp inoculum (20% moisture content) in sealed glass

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tubes for 12 h at 40°C in a water bath eliminated infectivity in all species. However, if inoculum was air dried and then placed uncovered in an oven at 55°C for 12 h viability dropped by about 70%. *G. mosseae* and *G. caledonium*, but not the other two species, were equally resistant to heating at 65°C for 12 h under the same conditions. These results indicate that these fungi are unusually resistant to heat. (Warner, Gee and Fyson)

Studies on the infectivity of fungal mycelium. It was observed that *Glomus mosseae* inoculum produced in sphagnum peat retained infectivity when milled (800 μm sieve plate) and passed through a 38 μm sieve, which is $\leqslant \frac{1}{2}$ the diameter of the resting spore. To test whether such infection resulted from fragments of infective root pieces or fungal mycelium, inoculum was produced by growing infected host plants within a fabric screen which did not allow growth of the roots into the peat surrounding the screen. Fungal mycelium grew through the screen and was harvested with the peat. The peat inoculum was air dried and passed through a 38 μm sieve, removing resting spores. This material, which did not contain infected root fragments or resting spores, was infective. (Warner, Gee and Fyson)

Biological nitrogen fixation

The carbon costs of N_2-fixation. A major factor contributing to the symbiotic performance of the legume/*Rhizobium* symbiosis is the efficiency with which carbon compounds provided by the host plant are used in N_2-fixation.

Methods based on the exposure of root nodules to $^{15}N_2$ and acetylene or argon, in a circulating gas system, have been used to evaluate this efficiency (see Witty, *Rothamsted Report for 1981*, Part 1, 215). Respiratory CO_2 production linked directly to nitrogenase can be distinguished from background respiration because of an interrelated decline in nitrogenase activity and respiration under conditions (for example in the presence of acetylene) which prevent the formation of the enzyme's normal end product, ammonia. Not all legumes exhibit this decline but where it is absent the effect can be induced by small decreases in oxygen concentration.

Indirect evidence suggests that legume/*Rhizobium* combinations which exhibit a decrease in respiration and nitrogenase activity under acetylene are distinguished by having a mechanism for rapidly altering the rate of O_2 flux into the nodule and it is this mechanism that causes the decrease. The nitrogenase activity of pea and clover nodules (which show a decrease in activity under acetylene) does not drop significantly at 80% O_2, whereas the activity of soyabean and sainfoin nodules (which do not show a decline in the presence of acetylene) falls to zero as O_2 damage occurs. The nitrogenase of both pea and clover can be partially inactivated if the rise in O_2 concentration is very rapid (*c.* 30 s) but this inactivation is avoided if the O_2 level is increased progressively over a 5 min period. Thus the O_2 tolerance would appear to be due to an induced decrease in permeability, which takes place over several minutes, rather than to any permanent structural feature.

The effects of plant age, plant species and *Rhizobium* strain have been investigated. The efficiency with which carbon is used by nitrogenase ranged, with field-grown *Vicia faba* nodulated by indigenous rhizobia, from 7–8·5 g C per g N fixed over the 4 month sampling period (May to September). Total root respiration increased until the final harvest but the proportion of this respiration which was coupled to nitrogenase declined from 58 to 15%. Similar results were obtained with cabinet grown peas but efficiencies ranged, over eight harvests, from 4·5–5·5 g C per g N fixed. A single harvest of glasshouse grown pea and *V. faba* plants nodulated by the same *Rhizobium* strain gave efficiencies

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of 5·7 and 7·0 g C per g N fixed respectively, demonstrating species-dependent differences in efficiency.

In 11 legume species tested efficiencies ranged from 3·4 (cowpea) to 8·3 g C per g N (sainfoin). With a single variety of pea, efficiencies varied with *Rhizobium* strain from 4·4 to 7·7 g C per g N. (Witty with Dr F. R. Minchin, Grassland Research Institute)

Tolerance of *Rhizobium* to heavy metals. The tolerance of 50 isolates of fast growing rhizobia to various heavy metal ions was investigated. Defined medium agar plates, each testing one heavy metal ion at a particular concentration, were inoculated with rhizobia by means of a multi-point inoculator. Each inoculation point was recorded after 96 h incubation at 28°C. The heavy metal ions could be ranked in order of decreasing toxicity as follows: Ag^{+}, Cu^{2+}, Hg^{2+}, CrO_4^{2-}, Cd^{2+}, Ni^{2+}, Co^{2+}, Zn^{2+}, Pb^{2+}, MoO_4^{2-}. No isolates were able to tolerate more than 0·05 μg Ag^{+} ml^{-1} in this medium; no growth inhibition by MoO_4^{2-} was detected at 1000 μg ml^{-1}. Patterns of tolerance to these metals were unique for 46 of the 50 isolates. Patterns were broadly similar for isolates of *Rhizobium leguminosarum*, *R. phaseoli* and *R. trifolii*. These were very different from those of *R. melilotti*. Isolates from *Lotus*, *Lupinus*, *Cytisus* and *Galega* were heterogeneous.

The heavy metal tolerance patterns of five *R. melilotti* isolates were examined in more detail. Tolerance to the metals used (Cu^{2+}, Cd^{2+}, Ni^{2+}, Co^{2+}, Zn^{2+}, Pb^{2+}) was reproducible for bacteria maintained on agar. Tolerance was tested for rhizobia from crushed nodules of *Medicago sativa* L. plants inoculated with these isolates. Variation in tolerance was increased but separation of all but one pair of the isolates was still possible. (Fyson, Gee and Warner)

The transfer of host range plasmids in *Rhizobium*. *R. leguminosarum*, *R. phaseoli* and *R. trifolii* are species of *Rhizobium* defined on the basis of the range of plants with which they can form nitrogen-fixing symbioses. The host range genes for these 'species' are extrachromasomal and transfer of the plasmid-borne genes is associated with the transfer of host range. An understanding of the genetics of host range in *Rhizobium* is important, because we want to be able to manipulate these bacteria to produce improved strains for inoculation purposes.

Many types of legume seed are treated with fungicides before sowing and some of these fungicides interfere with normal inoculation procedures because they are toxic to *Rhizobium*. Captan and captafol are particularly toxic, which suggests that resistant strains of *Rhizobium* should be selected to use on treated seed. Unfortunately all captafol-resistant derivations of *R. trifolii* strain 169 lost the ability to nodulate clover. To test whether captafol-resistance itself was responsible for the loss of symbiotic properties, captafol-resistant *R. trifolii* strains were crossed with *R. leguminosarum* and captafol-resistant bacteria carrying the *R. leguminosarum* host range genes were selected. All bacteria carrying the *R. leguminosarum* host range genes formed nitrogen-fixing nodules on peas, the normal host for *R. leguminosarum*. Thus captafol resistance does not necessarily prevent a strain from nodulating a legume. The effect of transferring a *R. trifolii* host range plasmid to the captafol-resistant strains and the range of interactions that occur when *Rhizobium* strains carrying different host range plasmids are inoculated on to different groups of legumes are being studied. (Dr J. E. Ruiz Sainz, University of Seville, Wadey and Beringer)

Plasmid transfer from *Rhizobium leguminosarum* to fast-growing soybean rhizobia. *Rhizobium* strains USDA 193 and USDA 194 are unusually fast growing for rhizobia. They were isolated in China and are able to form nitrogen-fixing nodules on some varieties of soybean. These bacteria have a number of attributes which could be useful

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for inoculants for temperate legumes. In order to develop an understanding of the genetics of these bacteria the host range plasmid PJB5JI from *R. leguminosarum* (which nodulates peas) was introduced into strains USDA 193 and USDA 194.

Pea seedlings inoculated with USDA 193 (PJB5JI) or USDA 194 (PJB5JI) developed small white root swellings similar to nodule initials. Sections of swellings induced by USDA 193 (PJB5JI) showed them to be small tumour-like growths with no cell differentiation. In a few cases there was enlargement, or swelling of the root vascular trace, but no branching was observed. Swellings induced by *R. japonicum* strain USDA 194 (PJB5JI) were similar, but there was no enlargement of the vascular trace. In two of the tumour-like growths the start of an endodermis was observed partially surrounding the swelling. There were no bacteria in any of the swellings sectioned to date.

No such swellings were observed in uninoculated controls, nor in seedlings inoculated with unaltered *R. japonicum* strains. (Chandler, Dr J. E. Ruiz Sainz, University of Seville, and Beringer)

Host and strain interactions in the *Rhizobium phaseoli* and *Phaseolus vulgaris* symbiosis. An important criterion in strain selection for inoculum production is the degree and nature of any host × strain interaction. The effect of plant genotype on competition for nodule formation by *R. phaseoli* strains and on the proportion of plant N derived from symbiosis has been studied in *R. phaseoli*-free soil in pot experiments.

In earlier field experiments done at Wellesbourne and Woburn there appeared to be little, if any, host × strain interaction. Pot experiments, using six strains of *R. phaseoli* on eight widely differing genotypes of dry seeded *P. vulgaris* confirmed the field observations.

The effect of host genotype on competition among five effective strains of *R. phaseoli* was also studied; plant genotype had no effect on the competitive hierarchy. In the same experiment ^{15}N labelled NH_4NO_3 was applied at low levels to both inoculated and uninoculated pots of each genotype. Indeterminate cultivars obtained a higher proportion of their N from symbiosis and proportionally less from the soil. (Day, Ewens and Stein)

Competition among strains of *Rhizobium phaseoli* for nodule formation. Five strains of *R. phaseoli*, two of tropical origin obtained from the Centro Internacional de Agricultura Tropical (CIAT) collection and three from the UK were tested for their abilities to compete with each other over a range of root temperatures, soil pH conditions and soil types. At all soil pHs tested on several soil types the tropical strains were more competitive than those of temperate origin at near optimum temperatures for plant growth (*c.* 27°C). At lower temperatures 21°C and 24°C the temperate strains were more competitive than they were at 27°C but still in a minority. As root temperature increased there was a progressive decline in their representation until at 36°C they were absent. The latter observation has important implications for strain selection for the tropics. (Stein and Day)

Competition between strains of *Rhizobium leguminosarum* for nodulation of *Pisum sativum* cultivars. Last year (*Rothamsted Report for 1981*, Part 1, 213–214) we reported that differences in the competitive ability of *R. leguminosarum* strains were related to the genotype of the host plant. Major differences in competitiveness were observed when mixed inocula were added to *Pisum sativum*, *Vicia faba* or *Lens esculenta*. This year the effect of host genotype was studied using five different lines of peas and three strains of *R. leguminosarum*. Nodule occupancy was ascertained by immunofluorescence. While an effect of host genotype was observed, it was much less obvious than in the previous work

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when different species were used. However, the differences observed were sufficiently great to suggest that host preference may be a useful characteristic to select for when producing *Rhizobium* strains for inoculation purposes. (Bitanyi and Beringer)

Nitrogen fixation in tropical grain legumes. A series of field-based isotope dilution and 'A' value experiments are being conducted in collaboration with International Crops Research Institute for the Semi Arid Tropics (ICRISAT) to develop methods to quantify N_2-fixation in groundnut, chickpea and pigeon pea. Studies with chickpea and groundnut are aimed at screening germplasm to identify high fixing cultivar × *Rhizobium* strain combinations, using safflower and a non-nodulating line of groundnut as respective non-fixing control plants.

The measurement of nitrogen fixation and the selection of a non-fixing control plant for studies with pigeon peas has posed a unique set of problems. The pigeon pea is deep rooted, has a long growing period and there are no known non-nodulating cultivars. The analysis of xylem sap for ureides or total reduced N to NO_3^- ratios has been suggested as a method for measuring nitrogen fixation, but is not applicable to pigeon peas due to a high root NO_3^- reductase activity. By pulse labelling the roots with $^{15}NO_3^-$, fractionating and estimating total N and ^{15}N present in the xylem sap as ureide, amide plus amino acid and NO_3^- fractions, it is possible to determine the proportions of NO_3^- reduced in the roots and thus obtain an estimate of the proportion of the plant's N derived from biological nitrogen fixation at the time of harvest. This method however is only applicable to irrigated crops. (Day, Giller, Ewens and Davitt)

Associative N_2-fixation with sorghum and millet. This work forms part of a collaborative programme between ICRISAT and this Department, funded by ODA. Previous studies at ICRISAT have identified consistent differences in nitrogenase activity, as estimated by the acetylene reduction technique, in the rhizosphere of different cultivars of both sorghum and millet. We are using ^{15}N-based methods to quantify and assess the agronomic importance of N_2-fixation in the rhizosphere of these important crops. Two approaches are being employed; the exposure of roots systems to $^{15}N_2$ gas and the use of ^{15}N-labelled fertilizer.

Several experiments have been carried out in which $^{15}N_2/O_2$ (80:20) mixtures were introduced into root incubation chambers attached to a sealed gassing manifold. Incorporation of ^{15}N into the roots and shoots of sorghum seedlings was evident within 3 days and equilibrium of the fixed N throughout the plant was complete 7 days later, indicating a rapid transfer of the fixed N from the microsymbiont to host plant.

In the first isotope dilution experiment using ^{15}N-labelled fertilizer, lines of sorghum and millet were grown with nutrient solutions containing 10 ppm N at a constant ^{15}N enrichment; results indicated that up to 25% of plant nitrogen may be derived from fixation. (Giller, Day and Davitt)

Staff and visiting workers

K. Giller joined the Department in February to work on a 2 year project funded by the ODA to develop methods for measuring nitrogen fixation in the rhizosphere of cereals. Also in February Angela Davitt was appointed to work on another ODA-funded project, replacing Valerie Harju who resigned in October, 1981. G. Carr and Mary Hinkley joined the Department in October to work on a 1 year project funded by NRDC to study methods for growing VA mycorrhizal fungi in pure culture. Penny Hirsch was appointed as a permanent member of staff in December. She will be using her expertise in DNA studies to work with *Rhizobium* and VA mycorrhizal fungi.

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E. Bromfield resigned in October to take up a permanent position at Agriculture Canada in Ottawa. He was joined by Michele Stein, who had finished the practical work for her Ph.D.

We were fortunate to have two sandwich students working with us. Wyn Chan spent 6 months working on VA mycorrhizal fungi (April to September) and R. Wadey is spending a year in the Department (June, 1982 to June, 1983) working on *Rhizobium*.

The Department welcomed a number of visitors from overseas and has benefited from their work and new ideas. The following came to work on mycorrhizal fungi: E. Bååth (Sweden) 1 year; D. Kandasamy (India) 3 months; Maria Tavares (Brazil) 22 months; A. Schubert (Italy) 5 months. Others have worked on biological nitrogen fixation: J. Ruiz Sainz (Spain) 1 year; H. Sepetoglu (Turkey) 4 months; A. Balasubramanian (India) 6 months; E. Mahne (Jugoslavia) 1 month; and S. Chaudhry has joined us for 1 year to study methods of isolating and characterizing soil microorganisms.

Several members of staff attended conferences in the UK or abroad, or spent short periods working in overseas laboratories.

J. E. Beringer gave talks on biological nitrogen fixation and microbial genetics: Monsanto Company in St Louis (USA); The Fourth International Symposium on the Genetics of Industrial Microorganisms in Kyoto (Japan); The 60th Anniversary meeting of the Society of Fermentation Technology of Japan in Osaka (Japan); in research institutes in Peking, Wuhan and Shanghai during a visit to China; The XIII Nardiske Kongres for Plantefysiologi in Aarhus (Denmark); the University of Helsinki (Finland); The joint Annual Meeting of the Societa Italiana di Genetica Agraria, and the Associazione Genetica Italiana in Bordighera (Italy); The First International Symposium on the Molecular Genetics of the Bacteria Plant Interaction in Bielefeld (West Germany) and the University of Ghent (Belgium). Margaret E. Brown gave an invited lecture at the 13th International Congress of Microbiology in Boston (USA). J. M. Day returned from a 6 month secondment to ICRISAT (India) in May, 1982; he also presented invited papers at the IAEA Coordination Meeting on the Use of Isotopes in Grain Legumes in Vienna (Austria) and at an EEC Workshop on the Measurement of Nitrogen Fixation at Roskilde (Denmark). Anne Warner gave seminars at the University of Wyoming, Laramie and the University of Colorado, Fort Collins (both in the USA). J. F. Witty presented invited papers at the Second National Symposium on Biological Nitrogen Fixation, Helsinki (Finland) and at the EEC Workshop on the Measurement of Nitrogen Fixation, Roskilde (Denmark).

Publications

General Papers

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